FORM PTO-1390 U.S. Department of Commerce Patent and Trademark Office

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

Attorney's Docket No. 1181-258 #7

U.S. Application No. (if known, see 37 CFR 1.5) 10/069,613

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/03378	31 August 2000	31 August 1999

TITLE OF INVENTION: **METHODS OF PEPTIDE PREPARATION**

APPLICANT(S) FOR DO/EO/US: **Øystein REKDAL, John Sigurd SVENDSEN, Mari WIKMAN, Terese SOLSTAD and Nannan YANG**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [ ] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. [X] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [ ] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [ ] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

ITEMS 11. TO 20. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A **FIRST** preliminary amendment.
14. [X] A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information: Response to Notification of Defective Response: Copy of Notification of Defective Response; computer disk of Sequence Listing; and paper copy of Sequence Listing (21 pgs)

21. [X] The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492)(a)(1)-(5):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report Not Prepared by EPO or JPO ..... $ 1,040.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report has been prepared by the EPO or JPO ..... $ 890.00		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... $ 740.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but claims did not satisfy provisions of PCT Article 33(1)-(4) ..... $ 710.00		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) ..... $ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$	
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	36 -20 =	16	X $18.00	$	
Independent Claims	06 -03 =	03	X $84.00	$	
Multiple dependent claim(s) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL =				$	
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$	
				Amount to be refunded	$
				charged	$

a. ☐ Checks in the amount of $_____ to cover the above fees are enclosed.

b. ☐ Please charge my Deposit Account No. 02-2135 in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2135. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Customer No. 6449

Barbara G. Ernst
Rothwell, Figg, Ernst & Manbeck
555 13th St., N.W.
Washington, D.C. 20004
Phone: 202/783-6040

Barbara G. Ernst
Signature

Barbara G. Ernst
Name

30,377
Registration Number

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents, Box PCT
United States Patent and Trademark Office
Washington, D.C. 20231
www.uspto.gov

FEB - 7 2003

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/069,613	Oystein Rekdal BGE 1181-258	1181-258

INTERNATIONAL APPLICATION NO.	
PCT/GB00/03378	
I.A. FILING DATE	PRIORITY DATE
08/31/2000	03/09/2000

6449
ROTHWELL, FIGG, ERNST & MANBECK, P.C.
1425 K STREET, N.W.
SUITE 800
WASHINGTON, DC 20005

CONFIRMATION NO. 3472

371 FORMALITIES LETTER

OC000000009454724

Date Mailed: 02/03/2003

NOTIFICATION OF DEFECTIVE RESPONSE

The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as an Elected Office (37 CFR 1.495):

ENTERED 2/9/03

- U.S. Basic National Fee
- Priority Document
- Biochemical Sequence Listing
- Copy of references cited in ISR
- Copy of the International Application
- Copy of the International Search Report
- Oath or Declaration
- Preliminary Amendments

Applicant's response filed 07/18/2002 is hereby acknowledged. The following requirements set forth in the NOTIFICATION of MISSING REQUIREMENTS mailed 05/20/2002 have not been completed.

Applicant is required to complete the response within a time limit of ONE MONTH from the date of this Notification or within the time remaining in the response set forth in the Notification of Missing Requirements, whichever is the longer. No extension of this time limit may be granted under 37 CFR 1.136, but the period for response set in the Notification of Missing Requirements may be extended under 37 CFR 1.136(a).

The following items **MUST** be furnished within the period set forth below:

- The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821-1.825 for the following reason(s):
  - A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e).
  - A copy of the "Sequence Listing" in computer readable form has been submitted. The content of the computer readable form, however, does not comply with the requirements of 37 CFR 1.822 and/or 1.832, as indicated on the attached marked-up copy of the "Raw Sequence Listing."
  - APPLICANT MUST PROVIDE:
    - An initial or substitute computer readable form (CRF) of the "Sequence Listing."

- An initial or substitute paper copy or compact disc of the "Sequence Listing," as well as an amendment directing its entry into the specification.

- For questions regarding compliance to 37 CFR 1.821-1.825 requirements, please contact:
  - For Rules Interpretation, call (703) 308-4216
  - To Purchase PatentIn Software, call (703) 306-2600
  - For PatentIn Software Program Help, call (703) 306-4119 or e-mail at patin21help@uspto.gov or patin3help@uspto.gov

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

*A copy of this notice **MUST** be returned with the response.*

KAYA L LEWIS BALTIMORE

Telephone: (703) 305-3695

PART 1 - ATTORNEY/APPLICANT COPY

U.S. APPLICATION NUMBER NO.	INTERNATIONAL APPLICATION NO.	ATTY. DOCKET NO.
10/069,613	PCT/GB00/03378	1181-258

FORM PCT/DO/EO/916 (371 Formalities Notice)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
	Application No.	10/069,613
	Filing Date	July 18, 2002
	First Named Inventor	Oystein REKDAL
	Group Art Unit	1646
	Examiner Name	Not Yet Assigned
	Attorney Docket No.	1181-258
Title of the Invention: METHODS OF PEPTIDE PREPARATION		

RESPONSE TO NOTIFICATION OF DEFECTIVE RESPONSE AND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Notification of Defective Response dated February 3, 2003, (copy attached) and prior to examination on the merits, please enter the amendments contained on the following pages.

IN THE SPECIFICATION:

Substitute paragraphs in the specification are submitted as shown on the following pages.

IN THE SEQUENCE LISTING:

Please insert the attached initial Sequence Listing

Marked-up copies of the original text of the specification pages are attached to this amendment. Material inserted is indicated by redlining (redlining) and material deleted is indicated by strikeout (~~strikeout~~).

Clean Copy of Substitute Specification page 21, second full paragraph, bridging page 22:

For those peptides with smaller cationic sectors e.g. the 15 mer peptide KKWAKKAWKWAKKAW [SEQ ID NO: 27] which has only 7 residues forming the cationic sector as opposed to 9 residues in the 21 mer peptide described above, a greater degree of bulk and lipophilicity is desirable for optimum therapeutic activity and selectivity and four tryptophan residues present in the flanking regions gave excellent results. Thus there is a balance, if a peptide is highly cationic and thus has a very strong attraction for negatively charged phospholipids in the cell membranes, a smaller overall number of bulky and lipophilic groups are desirable for optimum selectivity or it may be necessary to place some of the bulk and lipophilicity in the less active regions, i.e. in the regions opposie the cationic sector, in order to reduce the impact of the bulky and lipophilic groups e.g. to reduce toxicity. If a molecule has fewer cationic residues, then it may be necessary to place all the bulky and lipophilic residues in the most active regions of the peptide adjacent to the cationic sector. The results and principles discussed herein enable the skilled man to optimise the activity and selectivity of his chosen peptide system.

Clean Copy of Substitute Specification page 24, third paragraph:

In the case of LFB(17-31), a 15 amino acid fragment of LFB having the sequence Phe-Lys-Cys-Arg-Arg-Trp-Gln-Trp-Arg-Met-Lys-Lys-Leu-Gly-Ala [SEQ ID NO: 34], non-essential amino acids determined using an alanine scan were Cys(3), Gln(7) and Gly(14), here the numbering is in absolute terms relating to the peptide itself. Analogs of LFB(17-31) wherein these amino acids are replaced by non-genetic bulky and lipophilic amino acids may be particularly effective. For modifications to magainin peptides such as magainin 2, incorporation of non-genetic bulky and lipophilic amino acids at positions Phe(16) and Glu(19) may be particularly effective.

Clean Copy of Substitute Specification page 34, eighth paragraph:

Figure 4(a) shows helical wheel projections of the $(KAAKKAA)_3$ [SEQ ID NO: 13] peptide and (b) the same peptide substituted by 3 tryptophan residues or (c) 4 tryptophan residues.

Clean Copy of Substitute Specification page 37, first full paragraph:

Example 1

The principles discussed herein were used in the design, synthesis and testing of peptides based on a perfectly amphipathic helical conformation comprising only alanine and lysine residues. The sequence of the starting peptide was as follows, KAAKKAA KAAKKAA KAAK [SEQ ID NO: 35] referred to as "KA18". Modifications to this peptide to introduce one or more bulky and lipophilic residues were made by substituting Ala in flanking sector positions 7, 9 or 16 or in opposite sector positions 6, 10 or 17. Helical wheel representations of the two tri-substituted KA18 peptides are shown in Figure 3.

Clean Copy of Substitute Specification page 38, Table 1:

Peptide	IC_{50} Math A μM	IC_{50} Fibroblast μM	EC_{50} RBC μM
KA $18W_{10}$ [SEQ ID NO: 36]	>234	>234	>467
KA$18W_{16}$ [SEQ ID NO: 37]	>234	>234	>467
KA $W_{7,16}$ [SEQ ID NO: 38]	>222	>222	>444
KA $18W_{6,10,17}$ [SEQ ID NO: 39]	>211	>211	>422
KA $18W_{7,9,16}$ [SEQ ID NO: 40]	32	>211	>422

Clean Copy of Substitute Specification page 38, second full paragraph:

Example 2

As a model peptide we chose an analogue of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. Based on the sequence 14-31 of bovine lactoferrin, this peptide was modified to give an ideal amphipathic helical structure with a narrow cationic sector. LFB (14-31)m is LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$ (full sequence PAWRKAFRWAWRMLKKAA [SEQ ID NO: 1]). In this study one, two or all three of the Trp residues in the sequence were replaced by other amino acids, and the antibacterial, antitumoral and hemolytic activities were measured, as well as ability to inhibit fibroblasts.

Clean Copy of Substitute Specification page 39, Table 2:

Table 2

substitution	Peptide	Meth A IC_{50} (μM) (4h)	Mic E-coli (μM)	Mic S. Aureus (μM)	RBC EC_{50} (μM)	Fibroblast IC_{50} (μM
LFB (14-31)m [SEQ ID NO: 1]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	6.6(J)	2/4	2	110	17
Alanine						
W3-A3 [SEQ ID NO: 2]	LFB 14-31$A_{2,3,6,10,17}F_7K_{16}L_{14}R_4$	24.1	6.9	4.6	>463	190
W9-A9 [SEQ ID NO: 3]	LFB 14-31$A_{2,6,9,10,17}F_7K_{16}L_{14}R_4$	16.2	4.6	2.4	382	46.3
W11-A11 [SEQ ID NO: 4]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	11.1	4.6	>1.2	278	46.3
W9,11-A9,11 [SEQ ID NO: 5]	LFB 14-31$A_{2,6,9,10,11,17}F_7K_{16}L_{14}R_4$	110.1	14.7	14.7	>489	>489
Isoleucine						
W3-13 [SEQ ID NO: 6]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	9	2/4	2/4	323	20
W9-19 [SEQ ID NO: 7]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	12	5	<1	155	26
W11-111 [SEQ ID NO: 8]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	6	2/5	<1	63.6	15
W9,11-19,11 [SEQ ID NO: 9]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	22	35	19	284	26
W3,9-13,9 [SEQ ID NO: 10]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	36	5	5	**>470**	108
W3,11-13,11 [SEQ ID NO: 11]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	16	2.5	5	**413**	45
W3,9,11-13,9,11 [SEQ ID NO: 12]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	47	2.5	10	>487	280

Clean Copy of Substitute Specification page 42:

Example 3

The peptides described in table 3 below were made and tested as described in the previous Examples.

The model peptide $(KAAKKAA)_3$ [SEQ ID NO: 13] has 9 lysine and 12 alanine residues and its amphipathic helical wheel configuration is shown in Fig. 4a. This de novo designed antimicrobial peptide with low mammalian toxicity was selected from the literature (Javadpour et al. J. Med. Chem. 1996, 39, 3107-3113.) The MICs for this peptide against E. coli and S. aureus were 8 μM whereas it exhibited no measurable activity against fibroblasts or human erythrocytes.

Clean Copy of Substitute Specification page 43, Table 3:

KA-peptide	Abbr.	Posit.	Meth A IC_{50}	Fib IC_{50}	RBC EC_{50}	MIC S. aur	MIC E. coli	Ic_{50} Fib/ MethA
2 W								
$(KAAKKAA)_3$ $W_{9,16}$ [SEQ ID NO: 14]	KA 7	2F	>222	>444	>444	150	5	
3 W								
$(KAAKKAA)_3$ $W_{7,9,16}$ [SEQ ID NO: 15]	KA 3_2	1+2F	28	>422	>422	15	5	>15
$(KAAKKAA)_3$ $W_{2,9,16}$ [SEQ ID NO: 16]	KA 5	3F	15	302	>422	20	5	20
$(KAAKKAA)_3$ $W_{6,10,17}$ [SEQ ID NO: 17]	KA 3_1	30	147	>422	>422	35	10	>3
$(KAAKKAA)_3$ $W_{2,3,20}$ [SEQ ID NO: 18]	KA15	1+2F	16	246	>422	10	7,5-	15
$(KAAKKAA)_3$ $W_{7,10,17}$ [SEQ ID NO: 19]	KA23	1F+20	110	>422	>422			>4
$(KAAKKAA)_3$ $W_{7,16,17}$ [SEQ ID NO: 20]	KA24	1+1F+1	29	>422	>422			>15
4 W								
$(KAAKKAA)_3$ $W_{7,9,14,16}$ [SEQ ID NO: 21]	KA 4	2+2F	5	30	>402	2,5	2,5-	6
$(KAAKKAA)_3$ $W_{2,3,20,21}$ [SEQ ID NO: 22]	KA19	2+2 YF	19	374	>402			20
$(KAAKKAA)_3$ $W_{2,9,16,20}$ [SEQ ID NO: 23]	KA 8	4F	4	23	>402	5	5	6
$(KAAKKAA)_3$ [SEQ ID NO: 13]	KA 6	40	18	>402	>402	20	7,5	>22
4 F								
$(KAAKKAA)_3$ $F_{2,9,16,20}$ [SEQ ID NO: 24]	KA17	4F	37	>429	>429	10	5-	>12

Bip								
$(KAAKKAA)_3$ $Bip_{9,16}$ [SEQ ID NO: 25]	KA27	2F	24	>429	>429			>18
18-mer								
KKAWKWAKKAWKWAKKA [SEQ ID NO: 26]	KA18	2+2F	8	115	>451			14
15-mer								
KKWAKKAWKWAKKAW [SEQ ID NO: 27]	KA 22	2+2F	30	>514	>514			>17
WKWAKKAWKWAKKAA [SEQ ID NO: 28]	KA21	2+2F	32	>530	>530			>17
WKWAKKAAKWAWKAA [SEQ ID NO: 29]	KA20	2+2F	140	307	>546			2
Ornithine								
$(OAAOOAA)_3$ $W_{7,9,14,16}$ [SEQ ID NO: 30]	KA14	2+2F	5	60	>424	5	7.5-10	12

Clean Copy of Substitute Specification page 44, last paragraph, bridging page 45.

The following peptides have also been made:

(KAAKKAA) $_3F_{7,9,14,16}$ [SEQ ID NO: 31]

(KAKKKAA) $_3F_{6,10,13,17}$ [SEQ ID NO: 32]

(KAAKKAA) $_3Bip_{10,17}$ [SEQ ID NO: 33]

The presence of lysine residues as the provider of cationic character is clearly not essential as a peptide wherein all the lysine residues are substituted by ornithine shows good activity. In fact, the shorter side chain of the ornithine residues has enhanced selectivity as compared to lysine.

REMARKS

In response to a notification of defective response dated February 3, 2003 (a response copy is attached), an initial Sequence Listing is submitted, and its entry into the application is respectfully requested. An initial computer-readable form of the Sequence Listing is also submitted, and it is hereby certified that the content of the Sequence Listing information recorded in the computer readable form is identical to the Sequence Listing written on paper and contains no new matter. The amendments to the specification have been made to properly include the sequence identifiers.

Respectfully submitted,

By Barbara G Ernst

Barbara G. Ernst
Attorney for Applicants
Registration No. 30,377
ROTHWELL, FIGG, ERNST & MANBECK, p.c.
Suite 800, 1425 K Street, N.W.
Washington, D.C. 20005
Telephone: (202)783-6040

Attachments: Marked-Up Copies of Specification Pages
A copy of CRF of the Sequence Listing
A copy of the Sequence Listing on paper

1181-258.prelim.amdt.wpd

Amended Substitute Specification page 21, second full paragraph, bridging page 22: Version with markings to show changes made

For those peptides with smaller cationic sectors e.g. the 15 mer peptide KKWAKKAWKWAKKAW [SEQ ID NO: 27] which has only 7 residues forming the cationic sector as opposed to 9 residues in the 21 mer peptide described above, a greater degree of bulk and lipophilicity is desirable for optimum therapeutic activity and selectivity and four tryptophan residues present in the flanking regions gave excellent results. Thus there is a balance, if a peptide is highly cationic and thus has a very strong attraction for negatively charged phospholipids in the cell membranes, a smaller overall number of bulky and lipophilic groups are desirable for optimum selectivity or it may be necessary to place some of the bulk and lipophilicity in the less active regions, i.e. in the regions opposie the cationic sector, in order to reduce the impact of the bulky and lipophilic groups e.g. to reduce toxicity. If a molecule has fewer cationic residues, then it may be necessary to place all the bulky and lipophilic residues in the most active regions of the peptide adjacent to the cationic sector. The results and principles discussed herein enable the skilled man to optimise the activity and selectivity of his chosen peptide system.

Amended Substitute Specification page 24, third paragraph: Version with markings to show changes made

In the case of LFB(17-31), a 15 amino acid fragment of LFB having the sequence Phe-Lys-Cys-Arg-Arg-Trp-Gln-Trp-Arg-Met-Lys-Lys-Leu-Gly-Ala [SEQ ID NO: 34], non-essential amino acids determined using an alanine scan were Cys(3), Gln(7) and Gly(14), here the numbering is in absolute terms relating to the peptide itself. Analogs of LFB(17-31) wherein these amino acids are replaced by non-genetic bulky and lipophilic amino acids may be particularly effective. For modifications to magainin peptides such as magainin 2, incorporation of non-genetic bulky and lipophilic amino acids at positions Phe(16) and Glu(19) may be particularly effective.

Amended Substitute Specification page 34, eighth paragraph: Version with markings to show changes made

Figure 4(a) shows helical wheel projections of the $(KAAKKAA)_3$ [SEQ ID NO: 13] peptide and (b) the same peptide substituted by 3 tryptophan residues or (c) 4 tryptophan residues.

Amended Substitute Specification page 37, first full paragraph: Version with markings to show changes made

Example 1

The principles discussed herein were used in the design, synthesis and testing of peptides based on a perfectly amphipathic helical conformation comprising only alanine and lysine residues. The sequence of the starting peptide was as follows, KAAKKAA KAAKKAA KAAK [SEQ ID NO: 35] referred to as "KA18". Modifications to this peptide to introduce one or more bulky and lipophilic residues were made by substituting Ala in flanking sector positions 7, 9 or 16 or in opposite sector positions 6, 10 or 17. Helical wheel representations of the two tri-substituted KA18 peptides are shown in Figure 3.

Amended Substitute Specification page 38, Table 1: Version with markings to show changes made

Peptide	IC_{50} Math A μM	IC_{50} Fibroblast μM	EC_{50} RBC μM
KA $18W_{10}$ [SEQ ID NO: 36]	>234	>234	>467
KA$18W_{16}$ [SEQ ID NO: 37]	>234	>234	>467
KA $W_{7,16}$ [SEQ ID NO: 38]	>222	>222	>444
KA $18W_{6,10,17}$ [SEQ ID NO: 39]	>211	>211	>422
KA $18W_{7,9,16}$ [SEQ ID NO: 40]	32	>211	>422

Amended Substitute Specification page 38, second full paragraph: Version with markings to show changes made

Example 2

As a model peptide we chose an analogue of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. Based on the sequence 14-31 of bovine lactoferrin, this peptide was modified to give an ideal amphipathic helical structure with a narrow cationic sector. LFB (14-31)m is LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$ (full sequence PAWRKAFRWAWRMLKKAA [SEQ ID NO: 1]). In this study one, two or all three of the Trp residues in the sequence were replaced by other amino acids, and the antibacterial, antitumoral and hemolytic activities were measured, as well as ability to inhibit fibroblasts.

Amended Substitute Specification page 39, Table 2: Version with markings to show changes made

Table 2

substitution	Peptide	Meth A IC_{50} (μM) (4h)	Mic E-coli (μM)	Mic S. Aureus (μM)	RBC EC_{50} (μM)	Fibroblast IC_{50} (μM
LFB (14-31)m [SEQ ID NO: 1]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	6.6(J)	2/4	2	110	17
Alanine						
W3-A3 [SEQ ID NO: 2]	LFB 14-31$A_{2,3,6,10,17}F_7K_{16}L_{14}R_4$	24.1	6.9	4.6	>463	190
W9-A9 [SEQ ID NO: 3]	LFB 14-31$A_{2,6,9,10,17}F_7K_{16}L_{14}R_4$	16.2	4.6	2.4	382	46.3
W11-A11 [SEQ ID NO: 4]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	11.1	4.6	>1.2	278	46.3
W9,11-A9,11 [SEQ ID NO: 5]	LFB 14-31$A_{2,6,9,10,11.17}F_7K_{16}L_{14}R_4$	110.1	14.7	14.7	>489	>489
Isoleucine						
W3-13 [SEQ ID NO: 6]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	9	2/4	2/4	323	20
W9-19 [SEQ ID NO: 7]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	12	5	<1	155	26
W11-111 [SEQ ID NO: 8]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	6	2/5	<1	63.6	15
W9,11-19,11 [SEQ ID NO: 9]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	22	35	19	284	26
W3,9-13,9 [SEQ ID NO: 10]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	36	5	5	**>470**	108
W3,11-13,11 [SEQ ID NO: 11]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	16	2.5	5	**413**	45
W3,9,11-13,9,11 [SEQ ID NO: 12]	LFB 14-31$A_{2,6,10,17}F_7K_{16}L_{14}R_4$	47	2.5	10	>487	280

Amended Substitute Specification page 42: Version with markings to show changes made

Example 3

The peptides described in table 3 below were made and tested as described in the previous Examples. The model peptide $(KAAKKAA)_3$ [SEQ ID NO: 13] has 9 lysine and 12 alanine residues and its amphipathic helical wheel configuration is shown in Fig. 4a. This de novo designed antimicrobial peptide with low mammalian toxicity was selected from the literature (Javadpour et al. J. Med. Chem. 1996, 39, 3107-3113.) The MICs for this peptide against E. coli and S. aureus were 8 μM whereas it exhibited no measurable activity against fibroblasts or human erythrocytes.

Amended Substitute Specification page 43, Table 3: Version with markings to show changes made

KA-peptide	Abbr.	Posit.	Meth A IC50	Fib IC_{50}	RBC EC_{50}	MIC S. aur	MIC E. coli	Ic_{50} Fib/ MethA
2 W								
$(KAAKKAA)_3\ W_{9,16}$ [SEQ ID NO: 14]	KA 7	2F	>222	>444	>444	150	5	
3 W								
$(KAAKKAA)_3\ W_{7,9,16}$ [SEQ ID NO: 15]	KA 3_2	1+2F	28	>422	>422	15	5	>15
$(KAAKKAA)_3\ W_{2,9,16}$ [SEQ ID NO: 16]	KA 5	3F	15	302	>422	20	5	20
$(KAAKKAA)_3\ W_{6,10,17}$ [SEQ ID NO: 17]	KA 3_1	30	147	>422	>422	35	10	>3
$(KAAKKAA)_3\ W_{2,3,20}$ [SEQ ID NO: 18]	KA15	1+2F	16	246	>422	10	7,5-	15
$(KAAKKAA)_3\ W_{7,10,17}$ [SEQ ID NO: 19]	KA23	1F+20	110	>422	>422			>4
$(KAAKKAA)_3\ W_{7,16,17}$ [SEQ ID NO: 20]	KA24	1+1F+1	29	>422	>422			>15
4 W								
$(KAAKKAA)_3\ W_{7,9,14,16}$ [SEQ ID NO: 21]	KA 4	2+2F	5	30	>402	2,5	2,5-	6
$(KAAKKAA)_3\ W_{2,3,20,21}$ [SEQ ID NO: 22]	KA19	2+2 YF	19	374	>402			20
$(KAAKKAA)_3\ W_{2,9,16,20}$ [SEQ ID NO: 23]	KA 8	4F	4	23	>402	5	5	6
$(KAAKKAA)_3$ [SEQ ID NO: 13]	KA 6	40	18	>402	>402	20	7,5	>22
4 F								
$(KAAKKAA)_3\ F_{2,9,16,20}$ [SEQ ID NO: 24]	KA17	4F	37	>429	>429	10	5-	>12

Bip								
$(KAAKKAA)_3\ Bip_{9,16}$ [SEQ ID NO: 25]	KA27	2F	24	>429	>429			>18
18-mer								
KKAWKWAKKAWKWAKKA [SEQ ID NO: 26]	KA18	2+2F	8	115	>451			14
15-mer								
KKWAKKAWKWAKKAW [SEQ ID NO: 27]	KA 22	2+2F	30	>514	>514			>17
WKWAKKAWKWAKKAA [SEQ ID NO: 28]	KA21	2+2F	32	>530	>530			>17
WKWAKKAAKWAWKAA [SEQ ID NO: 29]	KA20	2+2F	140	307	>546			2
Ornithine								
$(OAAOOAA)_3\ W_{7,9,14,16}$ [SEQ ID NO: 30]	KA14	2+2F	5	60	>424	5	7.5-10	12

Amended Substitute Specification page 44, last paragraph, bridging page 45: Version with markings to show changes made

The following peptides have also been made:

(KAAKKAA) $_3F_{7,9,14,16}$ [SEQ ID NO: 31]
(KAKKKAA) $_3F_{6,10,13,17}$ [SEQ ID NO: 32]
(KAAKKAA) $_3Bip_{10,17}$ [SEQ ID NO: 33]

The presence of lysine residues as the provider of cationic character is clearly not essential as a peptide wherein all the lysine residues are substituted by ornithine shows good activity. In fact, the shorter side chain of the ornithine residues has enhanced selectivity as compared to lysine.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents, Box PCT
United States Patent and Trademark Office
Washington, D.C. 20231
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
10/069,613	Oystein Rekdal	1181-258

INTERNATIONAL APPLICATION NO.	
PCT/GB00/03378	
I.A. FILING DATE	PRIORITY DATE
08/31/2000	03/09/2000

6449
ROTHWELL, FIGG, ERNST & MANBECK, P.C.
1425 K STREET, N.W.
SUITE 800
WASHINGTON, DC 20005

CONFIRMATION NO. 3472

371 WITHDRAWAL NOTICE

OC000000009090420

Date Mailed: 02/03/2003

WITHDRAWAL OF PREVIOUSLY SENT NOTICE

The Notice mailed on 10/21/2002 was sent in error and is hereby withdrawn. A corrected Notice is enclosed. The time period for reply runs from the mail date of the enclosed Notice. We apologize for any inconvenience this caused.

KAYA L LEWIS BALTIMORE

Telephone: (703) 305-3695

PART 3 - OFFICE COPY